Border areas between gastroenterology and nephrology

Doctoral (Ph.D.) theses

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1. ABBREVIATIONS

ACE	angiotensin-converting enzyme
ACR	albumin-creatinine ratio
APC	argon plasma coagulation
ARB	angiotensin II receptor blocker
CAI	Colitis Activity Index
CD	Crohn's disease
CDAI	Crohn's Disease Activity Index
CRC	colorectal cancer
CRF	chronic renal failure
DM	diabetes mellitus
ESR	erythrocyte sedimentation rate
GAVE	gastric antral vascular ectasia
GIT	gastrointestinal tract
H-B	Harvey-Bradshaw
HCT	hematocrit
HPLC	high-performance liquid chromatography
hs-CRP	high-sensitivity C reactive protein
HT	hypertension
IBD	inflammatory bowel disease
IT	immunoturbidimetry
MAU	microalbuminuria
MDRD	Modification of Diet in Renal Disease
OPP	oetrogen-progesteron product
PLT	platelet count
RBC	red blood cell
TG	triglyceride
TL	telangiectatic lesion
UC	ulcerative colitis
WBC	white blood cell

2. INTRODUCTION AND AIMS

1. For clinicians, estimation of activity in inflammatory bowel disease (IBD) represents a difficult problem. In previous studies, urinary albumin determined by an immunological method (immunoturbidimetry /IT/) has been found to be a useful and simple marker of IBD activity. We aimed to compare urinary albumin excretion by means of a new high-performance liquid chromatography (HPLC) method and IT in both groups of IBD patients (Crohn's disease /CD/ and ulcerative colitis /UC/) and to find associations of these parameters with clinical activity of the disease.

2. Microalbuminuria (MAU) is an early marker of diabetic nephropathy. It has been proved to be a risk factor for atherosclerosis, for cardiovascular diseases and mortality. It also predicts all-cause mortality. In previous studies, increased albuminuria has also been detected in individuals with different locations of cancers. There was no correlation between MAU and colorectal cancer (CRC) at all. We aimed to investigate the urinary albumin of the patients with CRC by means of two different methodologies (IT and HPLC) and to find correlations between the results and the stage of CRC.

3. Gastric antral vascular ectasia (GAVE) as a vascular malformation is a rare cause of upper gastrointestinal bleeding. It is an important antral lesion because it can cause an occult chronic or severe acute bleeding. In the most cases it can be seen in cirrhotic patients but it is also more common in patients with chronic renal failure (CRF) together with isolated telangiectatic lesions (TL). The most reliable treatment method is argon plasma coagulation (APC). We aimed to investigate the prevalence of GAVE, to describe its appearance in patients with CRF and its therapy using oestrogen-progesteron product (OPP).

3. PATIENTS AND METHODS

1. We studied 207 outpatients with IBD (107 with CD, 100 with UC) between August 2007 and September 2008. The diagnosis of IBD was confirmed on the basis of endoscopy and histology. We excluded patients who suffered from diseases, conditions or were using medications which can modify the rate of urinary albumin excretion. After selection, a cross-sectional study with 60 CD patients and with 57 UC patients was performed and these patients were treated by usual treatment in IBD. As controls, urine specimens were obtained from 22 healthy medical students and health care professional volunteers. Routine laboratory measurements and first morning urine samples analyses by means of IT and HPLC were carried out.

2. We studied 54 patients with newly-diagnosed CRC between September 2007 and September 2009. The diagnosis of CRC was confirmed on the basis of endoscopy and histology. We excluded patients who suffered from diseases, conditions and were using medications which can modify the urinary albumin excretion. We did not exclude patients with diabetes mellitus (DM), and/or with hypertension (HT) and patients were treating with angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARB) and/or diuretics. Finally a study with 38 CRC patients was performed. As controls, 36 volunteers were examined who were adjusted for age, DM, HT and also for taking ACE inhibitors, ARB and diuretics. Routine laboratory measurements and first morning urine samples analyses by means of IT and HPLC were carried out.

3. We analyzed the data of 8783 patients who underwent an upper gastrointestinal endoscopical examination between 1996 and 2008. We determined the number of patients with CRF and GAVE and summarized the observations with OPPs.

4. RESULTS

1. Patients with CD and UC had significantly higher albumin-creatinine ratio (ACR) determined by IT and HPLC than did the healthy controls ($p<0.05$).

In CD, HPLC-ACR was significantly higher than IT-ACR in the active and inactive groups, as well ($p<0.01$). Using both IT and HPLC, higher ACR was measured in patients with active CD compared with patients with inactive CD ($p<0.01$). In addition, inactive patients (classified according to the Crohn's Disease Activity Index /CDAI/ and the modified Harvey-Bradshaw /H-B/ Activity Index) had higher albuminuria (measured either with HPLC or IT) than controls ($p<0.001$ for all). ROC curve analysis was carried out to select an appropriate cut-off value to predict likelihood of the clinical disease activity. These cut-off values were determined for IT-ACR and also for HPLC-ACR (IT-ACR in men was 0.37 mg/mmol, in women 1.62 mg/mmol, HPLC-ACR in men 2.46 mg/mmol, in women 5.30 mg/mmol) and their sensitivity and specificity were identified for the modified H-B Activity Index and CDAI.

In patients with UC, only IT-ACR was significantly higher in patients with active compared to inactive UC diseases as judged by the modified H-B Activity Index ($p<0.01$). HPLC-ACR did not differ between active and inactive cases. When grouping UC patients according to activity upon the Colitis Activity Index (CAI), neither IT- nor HPLC-ACR differ between active and inactive cases, but by using the HPLC method significantly higher ACR could be detected compared to IT-ACR in both disease activity scores (the modified H-B Activity Index and CAI) ($p<0.01$).

The correlations between HPLC-ACR and disease duration was not significant neither in CD nor in UC. Similarly, no correlation was found between MAU and disease extent (Montreal E) in UC. On the other hand, in CD, significant correlation was detected between MAU and disease extent (Montreal L). Patients with Montreal L3 had higher HPLC-ACR [8.51 (4.29-12.58) mg/mmol] compared to Montreal L1 [3.59 (3.09-4.30) mg/mmol] or L2 [1.90 (1.63-2.36) mg/mmol] [$p<0.01$ for both, data given as median (25-75 percentile)]. Only five patients had L4 i.e. upper gastrointestinal tract (GIT) manifestation, these patients tended to have higher albuminuria than patients without upper GIT manifestation, but the difference was not statistically significant.

In CD, we found significant correlations between HPLC-ACR and some inflammatory markers i.e. white blood cell (WBC) count ($r=0.257$; $p= 0.047$) and erythrocyte sedimentation rate (ESR) ($r=0.262$; $p= 0.047$). The correlation between HPLC-ACR and high-sensitivity C

reactive protein (hs-CRP) was not significant. In UC, there was no significant correlation between HPLC-ACR and the above markers of systemic inflammation.

Stepwise linear regression analysis was carried out in patients with CD to find predictors of clinical activity scores (the modified H-B Activity Index and CDAI). We tested a model including duration of disease, platelet count (PLT), WBC, ESR, hs-CRP, urinary IT-ACR, and urinary HPLC-ACR as independent variables and the modified H-B Activity Index as a dependent variable. Independent predictors of the modified H-B Activity Index were duration of disease, hs-CRP and urinary HPLC-ACR (adjusted r^2=0.562; duration of disease: ß=0.542, p<0.001; hs-CRP: ß=0.423, p=0.001; urinary HPLC-ACR: ß=0.269, p=0.036). We also tested the same linear regression model using CDAI as a dependent variable. Independent predictors of the CDAI were duration of disease, hs-CRP and urinary HPLC-ACR (adjusted r^2=0.511; duration of disease: ß=0.536, p<0.001; hs-CRP: ß=0.341, p=0.010; urinary HPLC-ACR: ß=0.284, p=0.036). In addition, stepwise linear regression analysis with the suspected activity markers was carried out in patients with CD to find predictors of clinical activity scores. We tested a model including HPLC-ACR, ESR, hs-CRP, WBC, PLT as independent variables and the modified H-B Activity Index as a dependent variable. Independent predictor of the modified H-B Activity Index was HPLC-ACR (adjusted r^2=0.202; p<0.001). We also tested the same linear regression model including IT-ACR, ESR, hs-CRP, WBC, PLT as independent variables and the modified H-B Activity Index as a dependent variable. Independent predictor of the modified H-B Activity Index was IT-ACR (adjusted r^2=0.123; p<0.017). When we tested a model including HPLC-ACR, IT-ACR, ESR, hs-CRP, WBC and PLT as independent variables, independent predictor of the modified H-B Activity Index was HPLC-ACR (adjusted r^2=0.252; p=0.001). When we searched for the independent predictor of CDAI, we tested a model including HPLC-ACR, ESR, hs-CRP, WBC, PLT as independent variables. Independent predictor of the CDAI was HPLC-ACR (adjusted r^2=0.307; p=0.001). We also tested a linear regression model including IT-ACR, ESR, hs-CRP, WBC, PLT as independent variables and the CDAI as a dependent variable. Independent predictor of the CDAI was IT-ACR (adjusted r^2=0.234; p=0.015). When we tested a model including both HPLC-ACR and IT-ACR beneath ESR, hs-CRP, WBC and PLT as independent variables, independent predictor of the CDAI was HPLC-ACR (adjusted r^2=0.342; p=0.003).

No similar analysis was carried out in UC patients as we found no significant correlations between urinary HPLC-ACR, IT-ACR and clinical scores.

2. Patients with CRC had significantly higher ACR determined by IT and HPLC than did the controls adjusted for age, DM, HT, taking ACE inhibitors, ARB and diuretics ($p<0.05$). HPLC-ACR was significantly higher than IT-ACR in patients with CRC and in controls as well ($p<0.01$).

There were no correlations between ACR measured either with HPLC or IT and the stage of CRC or between ACR measured either with HPLC or IT and carcinoembrional antigen, CA19-9, ESR, hs-CRP, serum hemoglobin, iron, transferrin, transferrin saturation, ferritin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, total protein, albumin, total cholesterol, HDL-cholesterol, creatinine, estimated glomerular filtration calculated by the Modification of Diet in Renal Disease (MDRD) formula. In patients with CRC significant correlations could be detected between IT-ACR and red blood cell (RBC) count ($r=-0.546$; $p=0.001$), hematocrit (HCT) ($r=-0.389$; $p=0.030$) and blood urea nitrogen ($r=0.368$; $p=0.042$), as well. In addition, significant correlations were present between HPLC-ACR and RBC count ($r=-0.456$; $p=0.009$), LDL-cholesterol ($r=-0.411$; $p=0.022$) and triglyceride (TG) ($r=-0.524$; $p=0.002$) serum level. The correlation between ACR and RBC count was independent from age, renal function, iron, transferrin, transferrin saturation and ferritin level. There was no correlation between RBC count and renal function, serum iron level, vitamin B12 level, and hs-CRP. Significant correlations could be detected between RBC count and both MAU and folic acid level ($r=0.340$; $p=0.042$).

Stepwise linear regression analysis was carried out in patients with CRC. We tested a model including HCT, RBC count and renal function as independent variables and the IT-ACR as a dependent variable. Independent predictor of IT-ACR was RBC count ($\beta=-0.540$; $p=0.002$). We also tested a linear regression model using RBC count, LDL-cholesterol, TG and phosphate as independent variables and the HPLC-ACR as a dependent variable. Independent predictor of HPLC-ACR was TG ($\beta=-0.466$; $p=0.014$).

3. In our clinic, 8783 patients underwent an upper gastrointestinal endoscopical examination between 1996 and 2008. Out of these cases, 18 patients were diagnosed with GAVE (0.2%). This date is similar to the international prevalence data. Four of the 14 patients had CRF. Two patients were treated with APC, two patients with GAVE and diffuse localized TLs were treated with OPP which successfully decreased the blood transfusion demand.

5. DISCUSSION

1. MAU can be a marker of disease activity in IBD. The cause of MAU in IBD can be the increased citokin level provoked by systhemic inflammation. The increased citokin level can result in increased glomerular permeability. Using both IT and HPLC, higher ACR was measured in patients with CD compared with healthy volunteers, and higher ACR was measured in patients with active compared with inactive CD. Measurement of albuminuria using HPLC is a more sensitive method detecting the disease activity in CD than the immunological method (IT). Above the IT- and HPLC-ACR cut off values calculated by means of ROC curve analysis the likelihood of the clinical disease activity is high.

In UC, ACR is not a good marker of disease activity. Only IT-ACR was significantly higher in patients with active compared to inactive UC diseases as judged by the modified H-B Activity Index. Measurement of albuminuria using HPLC is a more sensitive method detecting the disease activity also in UC than the immunological method (IT).

The correlation between HPLC-ACR and disease duration was not significant neither in CD nor in UC. Similarly, no correlation was found between MAU and disease extent in UC. On the other hand, significant correlation was detected between MAU and disease extent in CD.

In CD, we found a significant correlation between HPLC-ACR and some inflammatory markers i.e. WBC count and ESR. The correlation between HPLC-ACR and hs-CRP in CD was not significant. In UC, there was no significant correlation between HPLC-ACR and the above markers of systemic inflammation.

In CD, by means of stepwise linear regression analysis when it included duration of disease, PLT, WBC, ESR, hs-CRP, urinary IT-ACR, and urinary HPLC-ACR as independent variables and the modified H-B Activity Index as a dependent variable, independent predictors of the modified H-B Activity Index were duration of disease, hs-CRP and urinary HPLC-ACR. When we also tested the same linear regression model using CDAI as a dependent variable, independent predictors of the CDAI were duration of disease, hs-CRP and urinary HPLC-ACR. When we tested a model including PLT, WBC, ESR, hs-CRP, urinary IT-ACR and urinary HPLC-ACR as independent variables and both activity indices (the modified H-B Activity Index and CDAI) as dependent variables, independent predictors of the activity indices were urinary HPLC-ACR and IT-ACR. No similar analysis was carried out in UC patients as we found no significant correlation between urinary HPLC-ACR, IT-ACR and clinical scores.

2. MAU can be a sensitive marker of CRC without any correlation with the stage of the disease. Measurement of albuminuria using HPLC is a more sensitive method detecting the CRC than the immunological method (IT). We tested a model including HCT, RBC count and renal function as independent variables and the IT-ACR as a dependent variable. Independent predictor of IT-ACR was RBC count. We also tested a linear regression model using RBC count, LDL-cholesterol, TG and phosphate as independent variables and the HPLC-ACR as a dependent variable. Independent predictor of HPLC-ACR was TG.
We found no correlation between MAU and classical inflammatory markers in patients with CRC, which means that in these patients development of MAU does not only depend on inflammatory mechanisms.

3. GAVE as a vascular malformation is a rare cause of upper gastrointestinal bleeding. It is an important lesion because it can cause an occult chronic or severe acute bleeding. This lesion is relatively common in patients with CRF. In these patients this lesion can be seen with diffuse TLs and OPP can be used (instead of endoscopical and surgical methods) to reduce blood transfusion demand.

6. THESES

1/1. We justified that microalbuminuria is a simple and useful marker in disease activity of Crohn's disease.

1/2. In Crohn's disease, we found a significant correlation between microalbuminuria and disease extent, but no correlation can be found between microalbuminuria and disease duration.

1/3. We found that high-performance liquid chromatography is more sensitive than immunological method in detection of disease activity of inflammatory bowel diseases.

2/1. We justified that microalbuminuria is a sensitive marker of the presence of colorectal cancer.

2/2. We detected no correlation between microalbuminuria and the stage of colorectal cancer.

2/3. We found that high-performance liquid chromatography is more sensitive than immunological method in detecting of microalbuminuria in colorectal cancer.

3/1. We found that gastric antral vascular ectasia and diffuse localised teleangiectatic lesions in patients with chronic renal failure can be successfully treated by means of oestrogen-progesteron products. They can reduce the blood transfusion demand.

7. PUBLICATIONS OF THE AUTHOR

1. Publications on which the theses were based

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